HED DOC. NO. 013032

December 22, 1998

MEMORANDUM

SUBJECT: ***MALATHION:*** - **RE-EVALUATION** A Report of the Hazard Identification Assessment Review Committee.

FROM: Jess Rowland, Executive Secretary
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

THROUGH: K. Clark Swentzel, Chairman,
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Diana Locke, Risk Assessor
Reregistration Branch II
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PC Code: 057701

On November 6, 1997, the Health Effects Division's Hazard Identification Review Committee evaluated the toxicology data base, selected doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure risk assessments, and addressed the sensitivity of infants and children from exposure to malathion as required by the Food Quality Protection Act (FQPA) of 1996. The HIARC's conclusions were presented in the committee report issued on December 17, 1997 (*Memorandum:* J. Rowland to A. Nielsen, HED Document No. 012440)

Following that meeting, the Agency pursued the external peer review mechanism to address a number of issues raised by Dr. Brian Dementi, the malathion, toxicologist following the November 6, 1997 HIARC meeting. This peer review was conducted by soliciting comments from three experts in toxicology chosen by the Agency. The external peer review panel submitted their responses to the Agency in May, 1998. On August 18, 20 and 27, 1998, the HIARC evaluated the comments and responses provided by the external peer review panel.

These responses, the HIARC's evaluation of the panel's responses and the HIARC's

conclusions are presented in this report.

Committee Members in Attendance

Members in attendance were:

William Burnam
Robert Fricke
Karen Hamernik
Susan Makris
Melba Morrow
Kathleen Raffaele
John Redden
Jess Rowland (Executive Secretary)
Clark Swentzel (Chairman)

Data was presented by Brian Dementi of Toxicology Branch 1.

HED staff also in attendance at this meeting were:
E. Budd
S. Dapson
C. Jarvis
M. Lamont
A. Protzel
B. Tarplee
P. Wagner.

Report Preparation: _____

Jess Rowland
Executive Secretary

A. INTRODUCTION

On November 6, 1997, the Health Effects Division's Hazard Identification Review committee evaluated the toxicology data base to select the doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure risk assessments, and addressed the enhanced sensitivity of infants and children from exposure to malathion as required by the Food Quality Protection Act (FQPA) of 1996. The HIARC's conclusions were presented in the committee report issued on December 17, 1997 (*Memorandum:* J. Rowland to A. Nielsen, HED Document No. 012440)

Following that meeting, the Agency pursued the external peer review mechanism to address a number of issues raised by Dr. Brian Dementi, the malathion, toxicologist following the November 6, 1997 HIARC meeting. This peer review was conducted by soliciting comments from three experts in toxicology chosen by the Agency. The external peer review panel submitted their responses to the Agency in May, 1998 **(Attachment I)**.

On August 18, 20 and 27, 1998, the HIARC evaluated the comments and responses provided by the external peer review panel which are presented in Appendix I.

B. BACKGROUND

The external peer review panel (referred to henceforth as the Panel) consisted of three experts in toxicology selected by the Agency: Drs Michale Dourson, Rolf Hartung and Walter Decker. On behalf of OPP, Dr. Brian Dementi of Toxicology Branch 2, drafted a set of questions for the Panel under eight major topics. The Panel received all pertinent reference materials, namely the Data Evaluation Records of the toxicology studies, the One-Liner database and Dr. Dementi's memoranda and set of questions. The eight specific topics identified by Dr. Dementi are presented below. The specific questions for these topics are presented in Section III. HIARC's Evaluation of External Peer Review Panel's Response.

I. Hazard Identification/Acute Oral (One-Day)

II. Determination of Susceptibility, Reproductive Toxicity

III. Hazard Identification/Chronic Dietary (RfD)

IV. Subchronic Inhalation Study

V. Acute Neurotoxicity Study (Retinal Rosettes)

VI. Subchronic Neurotoxicity Study

VII. Cholinesterase Inhibition - Enhanced Sensitivity of Females

VIII. Cholinesterase Inhibition - Chronic Dog Study

Prior to the meeting, individual members of the HIARC with expertise in the areas of topics listed above were assigned to review the Panel responses and present their findings to the Committee. Dr. Dementi presented an overview of the Panel comments and guided the Committee through each topic. The Committee evaluated the Panel' responses and the assessments by the individual HIARC member assigned for each topic in conjunction with the malathion toxicology database.

III. HIARC's EVALUATION OF EXTERNAL PEER REVIEW PANEL'S RESPONSES'

Presented below are the questions presented to the Panel for each topic, a synopsis of the Panels responses and the HIARC's conclusions.

I. Hazard Identification/Acute Oral (One-Day)

Question 1): *Do the rabbit developmental toxicity and developmental range-finding toxicity studies support a conclusion that a single oral dose of malathion as high as 50 mg/kg would be without toxicological consequence in either the maternal or the developing organism?*

Panels Response: The Panel did not think the Agency's acute dietary endpoint of 50 mg/kg was justified based on the rabbit data and thought that an acute oral study measuring cholinesterase would be better.

HIARC's Conclusion: The Committee concluded that based on the combined results of the Range-Finding and Main Rabbit development study, a single oral dose of 50 mg/kg could be estimated to have no toxicological effect (i.e.,NOAEL) and thus is appropriate for acute dietary risk assessment. This dose was selected from a compilation (synthesized) of studies and is considered to be conservative for a single exposure (acute) dietary risk assessment.
The rationale for sustaining 50 mg/kg/day as the NOAEL for acute RfD is as follows: In the Range-Finding study no deaths occurred at 100 mg/kg/day. Death attributable to a single dose (i.e., the period of exposure of concern) occurred only in 1 doe on GD7 at 400 mg/kg/day and in does at 200 mg/kg/day after multiple doses (i.e., gestation days 11 and 17). Clinical signs seen in both studies were not attributable to a single dose. In the Main Study, the LOEL of 50 mg/kg/day was based on decrease in mean body weight gains in does during the dosing period. This decrease in mean body weight gains was not attributable to a single dose but rather to multiple doses. It should be noted no mortalities, clinical signs or decreases in body weight gain were seen when the same dose was tested in the Range-Finding study. Thus, toxicological endpoints (e.g., death, clinical signs, or certain developmental abnormalities) attributable to a single dose were not observed in does at 50 mg/kg/day.

Also, this dose was selected after review of the other oral studies (which are suitable for use in this risk assessment) that had much higher NOELs/LOELs such as the acute neurotoxicity study in rats (NOEL=1000 mg/kg/day, LOEL = 2000 mg/k) and the developmental toxicity study in rats (maternal NOEL=400 mg/kg/day, LOEL=800 mg/kg/day, developmental NOEL=>800 mg/kg/day). In particular, the acute neurotoxicity study in rats was not useful since cholinesterase data in this study showed much variation and a poor dose response relationship and thus was not appropriate for a regulatory endpoint.

Question 2): *Do data on maternal body weights and body weight gain now available in App. III of the rabbit development toxicity study, alter the assigned LOEL/NOEL for the study and does it influence the interpretation as to whether a single dose of malathion of 50 mg/kg would be without toxic effect?*

Panels Response: The panel was not influenced by the new data but thought it showed slight toxic effect at 50 mg/kg, but data were not relevant for single exposure at this dose..

HIARC's Conclusion: **The HIARC, again based on the weight-of-evidence of the data base (see rationale above for question 1), reaffirmed its original conclusion that 50 mg/kg/day is appropriate for acute dietary risk assessment.**

$$\textbf{Acute RfD} = \frac{\text{50 mg/kg/day (NOEL)}}{\text{100 (UF)}} = \textbf{0.5 mg/kg/day}$$

***Question 3)*:** *As presented in a published work in the open literature, a single intraperitoneal dose as low as 50 mg/kg/day in the rat reportedly elicited a clear effect on avoidance performance while cholinesterase inhibition (erythrocyte) was observed at 100 mg/kg. Plasma and brain cholinesterase were also inhibited at 150 mg/kg. Cholinesterase inhibition and decrements in behavior were all very significant though transient effects: a) What level of confidence should be accorded this study?; b) What is the implication of the route of administration to the question of whether a single oral dose of 50 mg/kg serve as an endpoint for acute dietary (one-day) risk assessment?; c) Is the data available in the developmental toxicity studies sufficiently reliable to discount the 10x safety factor required under FQPA?.*

Panel's Response: One member accorded low level of confidence to the intraperitoneal (i.p) study because i.p cannot be directly compare to relevant real-life exposure scenarios. The second stated that the intraperitoneal route is of questionable surrogacy for realistic environmental exposures. While, the third member reported that the study has the advantage of testing a relevant effect, he also stated that the route of exposure is an issue.

HIARC's Conclusion: The HIARC considered this route to be not appropriate for acute dietary risk assessment.

II. <u>Determination of Susceptibility, Reproductive Toxicity</u>

<u>Question 1)</u>*: Can the evidence indicating greater sensitivity of offspring versus parental animals in the two-generation reproduction study in the Sprague-Dawley rats be dismissed as ".....not a true indication of increased sensitivity of offspring....." for the reasons stated in the Hazard ID Committee report?.*

<u>Panels Response:</u> Two panel members stated that there is evidence indicating greater sensitivity (with qualifying remarks) while one stated that there is no indication for greater sensitivity

<u>HIARC's Conclusion:</u> In the two-generation reproduction study, for parental systemic toxicity, the LOEL was 7500 ppm (612 mg/kg/day in males and 703 mg/kg/day in females) based on decreased body weights in F_o generation during gestation and lactation and decreased body weight in F_1 during pre-mating. For parental systemic toxicity, the NOEL was 5000 ppm (394 mg/kg/day in males and 451 mg/kg/day in females).

The HIARC concurred with the NOEL/LOEL established by the reviewer in the Data Evaluation Record and reaffirmed the initial conclusion that the adult body weight gain data are confirmation of parental toxicity although it is recognized that the weight-of-evidence is <u>not</u> strong since there is lack of concordance between generations, and because the dose response is not pronounced. Nevertheless, the body weight decrements in F_0 females during gestation and lactation are valid and related; the weight decrements established in gestation are maintained during lactation, and can be attributed to maternal toxicity rather than to factors related to the pregnancy, such as litter size or weight.

The lack of a "significant" body weight gain difference during lactation is not sufficient evidence to discount the statistically significant decreases in mean body weight that were observed. Although the decreased body weight values of F_1 males, without concurrent body weight gain deficits, are not strong evidence of toxicity since F_1 weanling pups were significantly smaller, it was also noted that the males did not regain any of the weight deficits initiated in early life. If there were a total lack of parental toxicity at the highest dose tested, the body weight gains of the males may have demonstrated some recovery. Also, it was noted that the body weight data of F_1 females also indicate significant body weight decrements on weeks 1, 8, and 11, but not week 4 (other weeks were not reported). Therefore, the overall conclusion of the Committee was that parental toxicity was demonstrated by the body weight decrements observed.

It was also noted that the treatment level at which parental body weight decrements were observed was substantially (10-fold) greater than the treatment levels at which cholinesterase inhibition was seen in the chronic rat study with malathion. Although cholinesterase measurements are not recommended by the guidelines, and were therefore not performed, it is assumed that cholinesterase inhibition was indeed occurring in the parental animals which were maintained on test substance for at least 10 weeks premating and through approximately 8 additional weeks of reproductive life. This assumption is made because of cholinesterase inhibition observed in subchronic (13-weeks of dietary administration) and chronic studies with rats.

For offspring toxicity, the NOEL was 1700 ppm (131/153 mg/kg/day in males and females) and the LOEL was 5000 ppm (394 mg/kg/day in males and 451 mg/kg/day in females) based on decreased F_{1a} and F_{2b} pup body weights during lactation.

At the November 7, 1997 meeting it was determined that even though the offspring NOEL (131/153 mg/kg/day in M/F) was lower than the parental systemic toxicity NOEL (394/451 in M/F), this was not a true indication of increased susceptibility since: (i) pup body weight decrements were primarily seen at postnatal day 21; (ii) they are likely related to higher consumption of treated feed in late lactation; (iii) there is an assumption that malathion was present in the milk; and (iv) the pups were exposed to the compound both via the feed (at a high relative intake level) and the milk during late lactation, and were receiving an exaggerated dose of the test substance.

The, HIARC reaffirmed its previous conclusion that there is no increased susceptibility and that even though "quantitatively" there appears to be increased susceptibility based on the NOELs/LOELs. "Qualitatively" the "apparent" susceptibility is due to the assumed higher consumption of treated feed in late lactation and the assumed presence of malathion in the milk. The presence of the chemical in the milk is a generic assumption made during hazard assessment for all chemicals (unless we have data to show otherwise), and is not unique for malathion.

Under the current HED Standard Operating Procedures, the HIARC is not responsible for determining the retention, reduction or removal of the 10x safety factor. That determination was made by the FQPA Safety Factor Committee on June 15, 1998. The FQPA Safety Factor Committee evaluated the hazard and exposure (dietary, drinking water and residential) data and concluded that the 10x safety factor for the protection of infants and children (as required by FQPA) should be removed due to 1) completeness of the toxicology database; 2) lack of increased susceptibility in developmental and reproductive toxicity studies; and 3) the use of adequate data (actual, surrogate, and/or modeling outputs) to satisfactorily assess dietary exposure and screening level drinking water as well as residential exposure assessment.

Question 2): *In the absence of assessments of cholinesterase inhibition and behavioral effects testing in adult and young animals in reproduction studies, can the data obtained in the FIFRA guideline study be considered adequate to address the question of whether young or mature animals are more sensitive to malathion?.*

Panel's Response: The panel appears to agree in saying no to this question, i.e., data in the 2-generation reproduction study are not adequate to address the question of relative sensitivity of younger versus mature animals.

HIARC's Conclusion: The adequacy of the two-generation reproduction study to assess increased susceptibility is a generic issue, applicable to all chemicals, and not specific to malathion. At present the determination of susceptibility is made not based on the results of one study but rather on a weight-of-evidence basis that includes acute and subchronic neurotoxicity studies, the prenatal developmental

toxicity studies in rats and rabbits, the 2-generation reproduction toxicity study in rats as well as the toxicity profile of the chemical. The HIARC, in previous deliberations, has determined that, based upon the weight of the evidence, a developmental neurotoxicity study (which assesses behavioral effects in the offspring, as well as many other endpoints, and could potentially include cholinesterase inhibition for perinatal animals) would not be required for malathion at this time.

Question 3): *Does this two-generation reproduction study provide the reliable evidence of no increased sensitivity in pups when compared to adults, as required under FQPA, to discount the 10x safety factor imposed by FQPA as additional protection for infants and children?*

Panel's Response: One panel member suggested a 3x safety factor as opposed to 10x, while acknowledging that the 10x may still be useful as a management tool. The other two panel members said no, though, one member argued that the offspring must be shown to be less sensitive.

HIARC's Conclusion: The HIARC determined that the two-generation reproduction study submitted in support of malathion reregistration provided adequate and reliable data regarding reproductive toxicity and offspring effects, according to Agency guideline recommendations (83-4) and Good Laboratory Practices. The hazard and dose-response assessments are considered by the FQPA Safety Factor Committee along with the dietary (food and water) as well as residential exposure assessment during risk characterization in order to arrive at a determination of whether or not to recommend retention of the 10x FQPA Safety Factor. **This determination cannot be made based upon the hazard assessment of a single toxicity study**.

III. Hazard Identification/Chronic Dietary (RfD)

Question 1): *Given the evidence of a post 3 month recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, can 50 ppm be concluded to have been a NOEL for the first three months of testing?*

Question 2): *Alternatively, do these findings suggest flawed cholinesterase methodology, and if so, what corrective measure could be pursued?*

Question 3): *Should 4 mg/kg/day, the NOEL for plasma cholinesterase inhibition in males, be supported as a replacement for human data previously relied upon in establishing the RfD, or should additional testing be required in the rat to identify a NOEL for cholinesterase inhibition, particularly in females?*

Question 4): *Given that an explanation exists for greater sensitivity of humans than rats with respect to cholinesterase inhibition from malathion exposure (i.e., the lack of carboxylesterase in human plasma) should a 10x safety factor applied to the rat data to allow for "uncertainties" in inter-species variability be considered adequate if the rat data is to be used in deriving the RfD?*

Question 5): *Further, given the RfD based on human data (0.023 mg/kg/day) is lower than that derived from the rat data (0.040 mg/kg/day) and that an explanation exists for a greater sensitivity for humans, should the RfD based on human data be retained?*

Question 6): *Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10x safety factor imposed under FQPA for the protection of infants and children?*

Panel's Response: In their responses to these six questions, the panel made several assertions, suggestions and recommendations with regard to: (i) establishing a NOEL for the first three months in the two-year rat study (*Question* # 1); (ii) the adequacy of the cholinesterase methodology (*Question* # 2); (iii) the need for additional testing to identify a NOEL for cholinesterase inhibition (*Question* # 3); (iv) the need for additional uncertainty factors to account for deficiencies (*Questio*n # 4); and (v) the discounting of the 10x factor (*Question* # 6).

With regard to question #5 whether the human study should be retained for deriving the RfD, two members said yes, the human study should be retained since human is the correct species of concern while the third member said no, *"the rat study appears to be a stronger basis for RfD than human work"* but advocated *"a 3-fold uncertainty factor to account for deficiencies in the database, principally because the critical effect was not monitored in the two-generation reproduction study in a potentially sensitive subgroup (i.e., young rats)"*. This member also suggested that should the human study be retained, an additional uncertainty factor of "unspecified magnitude, probably less than 3, be applied" since human females were not tested.

HIARC's Conclusion: The HIARC reaffirmed its decision to derive the chronic RfD based on the NOEL of 4 mg/kg/day established in the combined chronic toxicity /carcinogenicity study in rats and the use of a UF of 100 to account for inter-species extrapolation and intra-species variation. The RfD remains at 0.04 mg/kg/day.

The HIARC concluded that the human study is not appropriate based on the following factors: (i) there is the low confidence in the human study because of possible confounding factors (e.g., smoking), the purity of malathion is unknown, and the raw data is unavailable for proper evaluation (published in 1962 in open literature); (ii) purity of malathion tested in the animal study is known (97.1%); (iii) the NOEL in the two-year rat study is supported by the NOEL of 4 mg/kg/day established in the subchronic neurotoxicity rat study (based on inhibition of cholinesterase activity); and (iv) the animal toxicology data base is complete except for the subchronic feeding study in dogs and an subchronic inhalation toxicity study in rats.

The HIARC also concluded that an no additional uncertainty factors are necessary since: (i) a NOEL (not a LOEL) was used to derive the RfD; (ii) this NOEL is supported by the same NOEL in the subchronic study in the same species (rats) for the same effects (cholinesterase inhibition) indicating no cumulative toxicity response over time; (iii) the RfD of 0.04 mg/kg/day derived using an animal study with a UF of 100 (for inter-species extrapolation and intra-species variation) is comparable to the RfD

of 0.02 mg/kg/day that can be derived by the use of the NOEL of 0.23 mg/kg/day from a human study and a UF of 10x for intra-species variation.

IV. Subchronic Inhalation Study

Question 1): *Is the use of a UF (uncertainty factor) of 3 to compensate for the absence of a NOEL for cholinesterase inhibition and nasal and laryngeal degeneration/hyperplasia supportable?*

Panel's Response: One member recommended against the use of additional UF, another, recommended a UF of 10, while the third member did not feel qualified to answer this question.

HIARC's Conclusion: The HIARC concluded that a Margin of Exposure of 1000 is required for Short-, Intermediate- and Long-Term inhalation exposures. The MOE of 1000 includes the conventional 100 and an additional 10 for the use of a LOEL and the severity of the nasal lesions.

This decision was based on the results of a two-week range finding study (MRID No. 44554301) which was not available to the Committee at the November 6, 1997 meeting. In that study, there was a dose-related increase in the lesions of the nasal cavity (hyperplasia and respiratory epithelium) which was similar to the laryngeal and nasal cavity lesions seen in the subchronic study.

Question 2): *A two-week range-finding inhalation study, evidently not available to the Hazard ID Committee, did not establish NOELs for cholinesterase inhibition or histopathology findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L. Should this study influence the Hazard ID Committee decision not to evoke an uncertainty factor for acute risk assessment (i.e., 1-7 days) on the basis of cumulative effects*?

Panel's Response: Conclusions from two members suggests that the cholinesterase inhibition is well characterized and that an extra UF is not warranted. The third member recommended against using this study since such studies (range finding) do not provide reliable information.

HIARC's Conclusion: The HIARC concluded that based on the availability of the new data (the range finding study), a MOE of 1000 is required also for Short-term inhalation risk assessment (previously it was determined that a MOE of 100 is adequate for this exposure period).

Question 3): *Should another study be required to identify the NOEL for the end points in question ?*

Panel's Response: One member would like to identify a NOEL, while the other suggests first using bench mark approach. The third does not want an inhalation study with rats.

HIARC's Conclusion: **The HIARC determined that a new inhalation study is required based on the results of the two-week range-finding study (MRID No. 44554301) and the lack of a NOAEL for cholinesterase inhibition in the 90-day study (MRID No. 43266601).**

Question 4)*: Given the findings of nasal and laryngeal degeneration/hyperplasia in both of the recently submitted malathion and malaoxon combined chronic toxicity/carcinogenicity studies and the finding of rare nasal tumors in the malathion study, should the Agency require a carcinogenicity study by the inhalation route (e.g., inhalation exposure for first 90 days of a two year study)?*

Panel's Response: One member said yes to requiring this study, another member does not want this study and the third member would like to see mode of action studies to understand nasal injury and questions the utility of the inhalation study.

HIARC's Conclusion: At its meetings held on September 24, October 8 and October 15, 1997, HED's Cancer Assessment Committee (CARC) determined that in order to conduct an accurate assessment on the relevancy of nasal tumors to malathion exposure, the nasal tissues from all animals from all dose groups in the 2-year carcinogenicity study (MRID No. 43942901) should be evaluated/re-evaluated (*Memorandum:* J. Rowland, to M. Ioannou, dated 11/3/97; HED Document No. 012374). **Therefore, the HIARC concluded that the need for a study will be determined after CARC's review and evaluation of the requested histopathological examinations.**

Question 5): *Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10x safety factor imposed under FQPA for the protection of infants and children?*

Panel's Response: The panel agreed that the study does not provide any support for discounting use of the 10x safety factor imposed by FQPA. One member acknowledged that the study does not evaluate young individuals and asserted that the FQPA 10x factor is a risk management tool and including it in the scientific discussion of database sufficiency is not appropriate.

HIARC's Conclusion: This study is not appropriate for FQPA assessment because: (i) the study was conducted in adult animals; (ii) there was no exposure to pregnant animals nor was there pre/post natal exposure; (iii) this study did not evaluate parameters in fetuses or pups; and (iv) is not appropriate for assessment of increased susceptibility under FQPA provisions.. **Therefore, HIARC concluded that discussion about the FQPA Safety Factor is neither applicable nor appropriate for this study. In addition, the FQPA Safety Factor, when required, is not applied to any single toxicity study but rather for dietary and residential exposure risk assessments.**

V. Acute Neurotoxicity Study (Retinal Rosettes)

Question 1): *Should retinal histopathology data be submitted for rats in the intermediate dose groups?*

Panel's Response: Two of the members said yes. The third member suggested that the decision to evaluate lower dose groups be made after re-evaluation of the slides in question.

HIARC's Conclusion: The HIARC noted that this issue of reexamination of the retinal tissue of three rats was addressed by an *ad hoc* subgroup of neurotoxicity experts in HED.

The *ad hoc* group met on November 13, 1997, and after careful evaluation of all available data, concluded that the Agency should not ask for evaluation of the retinal tissue of three rats in the acute and subchronic neurotoxicity studies. This decision was based on the following weight-of-evidence considerations (*Memorandum:* E. Budd to R. Loranger, dated December 3, 1997).

1) The lesion of concern (bilateral retinal rosette) occurred in only one male rat at the high dose in the acute neurotoxicity study.

2) A unilateral retinal rosette was also tentatively observed in one male rat in the control group in the subchronic neurotoxicity study.

3) Dr. Brennecke (HED's pathology consultant) and Dr. Dahlgreen (the study pathologist) both concluded the retinal rosette in the male rat at the high dose was not of toxicological significance and was not due to treatment with malathion

4) The *ad hoc* group also concluded that retinal rosettes in rats are most likely the result of abnormal proliferation and differentiation of developing retinal cells during neonatal life (i.e., during the first approximately 32 days after birth) and ordinarily are not likely to develop in mature animals as a result of treatment with xenobiotics.

5) An available reference (Ophthalmic Pathology of Animals, Saunders and Rubin, 1975), stated that "[retinal] rosettes occur spontaneously in certain strains of inbred rats and in beagle and collie dogs".

Based on this information, the HIARC differed with the Panel's recommendations and reaffirmed the *ad hoc* group's decision on this issue, concluding that no additional histopathological examination is necessary at this time.

Question 2): *Should histopathology slides be submitted for independent examination by the Agency's pathologist (for anatomic features comparisons between control and treatment group lesions) as called for in the Data Evaluation Record (DER) for this study (a relatively simple*

request)?

Panel's Response: All three members responded yes.

HIARC's Conclusion: The HIARC again differed with the panel and reaffirmed the decisions made by the *ad hoc* group based on the rationale provided above.

VI. Subchronic Neurotoxicity Study

Question 1 *Given the contrast between the NOEL of 1575 mg/kg/day (HDT) for female rats on neurotoxicity endpoints in this FIFRA Guideline study and that of the LOEL of 38 mg/kg/day (LDT) in the published work on a different set of neurotoxicity parameters, does the published work provide adequate reason or evidence to require a developmental neurotoxicity Guideline study, or another neurotoxicity study that embraces learning/memory , EEG, EMG, and possibly other neurotoxicity parameters not covered in the subchronic neurotoxicity Guideline study?*

Panel's Response: One panel member said yes. One member questioned the acceptability of the published study. The other member did not believe that the published study provided reason to require additional studies.

HIARC's Conclusion: The *ad hoc* group, after careful evaluation of all available data, concluded that the Agency should not ask for additional neurotoxicity studies on malathion at this time. It was recognized, however, that such studies might possibly be requested at some time in the future if there is sufficient justification for doing so. The group also suggested that additional literature searches should be conducted on learning/behavior effects of organophosphates in general, and available information on malathion particularly (*Memorandum:* E. Budd to R. Loranger, dated December 3, 1997).

The HIARC reaffirmed the *ad hoc* group's decision on this issue and concluded that no additional studies are required at this time. The HIARC also noted that lack of studies that evaluate learning and/or memory or behavioral effects under the Subdivision F Guideline requirement is a generic issue applicable to all organophosphates, and not particular to malathion. The HIARC recommended that the issue of requiring such a study should be evaluated in conjunction with discussion on the data requirements for FQPA.

Question 2): *If the neurotoxicity findings in the published study are considered inadequate to trigger the additional Guideline testing, what criteria from published work, short of those upon which regulations could be directly based, might serve in that capacity?. (Note: Moeller and Rider (1962), a journal publication with attendant Guideline deficiencies, has served for decades as the basis for a regulatable end point (RfD) for malathion, while the publication in question here is only being put forth as sufficiently definitive to require a study in the FIFRA Guidelines heretofore not performed).*

Panel's Response: One member deferred this question to Agency experts, the second member did not provide a response, and the third suggested having a neurotoxicologist provide criteria.

HIARC's Conclusion: As discussed above, the HIARC noted that this is a generic issue that needs further discussion by OPP.

VII Cholinesterase Inhibition - Enhanced Sensitivity of Females

Question 1): *Does the malathion data base support a conclusion that females are the more sensitive gender with respect to cholinesterase inhibition by this organophosphate?*

Panel's Response: One member says may be yes, but not in the two-year study used for establishing the RfD. The second member stated that the data are not presented in a proper manner for this assessment. The third member responded that yes, more data is needed to characterize the gender specific disparity.

HIARC's Conclusion: This issue (the possibility of greater sensitivity in one sex) has surfaced several times in the past with respect to setting RfD for other chemicals and, as a general policy, it has previously been decided that an additional uncertainty factor would not ordinarily be applied to the RfD based on possible sex-related differences

In considering sex related sensitivity to malathion, the entire data base should be examined to see if any peculiarities exist that could serve as a basis for claims of sex-linked sensitivity. If peculiarities are present, they should be further examined to determine whether they are consistent in their occurrence; affecting the same endpoint, and affecting females with the same degree of sensitivity across species lines.

The toxicology profile suggests that overall sensitivity to malathion is similar for both sexes and that there is no reason to believe that females are consistently more sensitive than males. In certain studies (e.g., subchronic neurotoxicity study in rats, the subchronic inhalation toxicity study in rats and the 21-day dermal toxicity study), females do indeed appear to be more sensitive than males as there are indications that the difference in cholinesterase inhibition is at least an order of magnitude when males were compared to females However, there is no clear picture on the relative degree of increased sensitivity of females compared to males when observed. When studies in which females appeared to be more sensitive are further examined to see what compartment of cholinesterase is affected, again there is no consistency. In some cases, the red blood cell and plasma activity appears to be indicators of sensitivity and in other cases, the brain cholinesterase activity appear to be more sensitive. Again, this finding is in studies in which females were designated as having lower NOAELs when cholinesterase was the endpoint of concern. In many (but not all) studies, the sex-related difference did not result in different cholinesterase NOELs for males and females, but rather in different degree of cholinesterase inhibition for males and females at a given dose level. The HIARC noted that NOELs, rather than degree of effect at a given dose level, are used to derive the RfD.

Question 2): *What approach might be taken to estimate, from the data currently available, a correction factor to be applied to the NOEL derived from the Moeller and Rider study in male human subjects to afford equivalent protection for women?.*
Panel's Response: The members were split on this issue and did not offer any concrete approach to this.

HIARC's Conclusion: The HIARC concluded that even if the human study (where no females were used) had been chosen as the basis for the RfD, it would not be appropriate to apply additional uncertainty factor to account for the increased sensitivity of females as compared to males. The rationale for this decision was that (i) when sex-related difference in sensitivity was observed, the difference appears to be small and (ii) the NOELs, rather than degree of effect are used to derive the RfD. However, the RfD is based on the chronic rat study, an additional factor based on sex would be of no relevance since the NOEL for plasma cholinesterase inhibition in that study was 50 ppm for both sexes (equivalent to 4 mg/kg/day in males and 5 mg/kg/day in females). (Note: one panel member also pointed out that the *"NOELs for cholinesterase inhibition in both male and female rats are the same in the critical study").*

Question 3): *Should additional testing in animal models be required to further quantitate the gender specific disparity?.*

Panel's Response: One member said no. Another suggested the study be extended to include females. The third member said yes, more data are needed to define gender disparity.

HIARC's Conclusion: It was the consensus of the Committee that additional testing is not necessary because: (i) the human study (with one sex) was not used for establishing the RfD; (ii) the NOELs for cholinesterase inhibition in both males and female rats are the same in the critical (animal) study used to derive the RfD (as duly noted by one of the Panel member); (iii) as discussed above, the "apparent" sex difference in sensitivity is not consistent across studies/species (some studies showed fairly large differences); and (iv) NOELs, rather than degree of effect at a given dose level, are used to derive the RfD.

VIII. Cholinesterase Inhibition - Chronic Dog Study

Question: *Knowing that the chronic dog study has no NOEL for cholinesterase inhibition and was considered unacceptable, should additional work, e.g., subchronic feeding study, be required to characterize cholinesterase inhibition in the dog?*

Panel's Response: Two members emphatically responded that no, another study is not required. The third member said yes, because a NOEL is required to comply with the guidelines.

HIARC's Conclusion: The HIARC concluded that a 90-day study in dogs is required based on the rationale provided below: (i) in 1988, the requirement for a subchronic feeding study in dog (§82-

1b) was waived contingent upon performance of a chronic toxicity study in dogs; (ii) in waiving this study, there is enhanced burden for the Registrant to provide an acceptable chronic study which was not achieved by the present study (MRID No.40188501); (iii) there are species-related biochemical similarities (absence of plasma carboxylesterase) to anticipate that the dog would respond similarly to man; (iv) since the chronic study was conducted in 1987, cholinesterase methodology may be problematic and should be examined for conformity with the most current Agency standards; (v) the contrast between doses inhibiting cholinesterase in man and in rat serves to indicate more definitive testing is required in a third species; and (vi) a subchronic study could possibly address the question of whether the type of dosing (capsule vs. dietary) is critical in the dog.

The HIARC recommended that the Registrant consult the Agency for study design and protocol prior to initiation of this study.

C. CONCLUSIONS

1. **No change in the dose and endpoint selected for deriving the acute RfD** at the November 6, 1997 HIARC meeting.

2. **No change in the dose and endpoint selected for deriving the chronic RfD** at the November 6, 1997 HIARC meeting.

3. **No change in the conclusion that there is no evidence of increased susceptibility** in the prenatal developmental toxicity studies in rats and rabbits following *in utero* exposure or in the pre/post natal two generation reproduction toxicity study in rats.

4. **A MOE of 1000 is required for Short, Intermediate and Long-term occupational/residential inhalation risk assessments** instead of a MOE of 100 for Short-term inhalation exposure risk assessment as recommended at the November 6, 1997 meeting.

5. **No additional retinal histopathological examination is required** from the acute neurotoxicity study.

6. **No additional neurotoxicity studies are required at this time**.

7. **The data does not provide a clear picture of enhanced sensitivity in adult females.**

8. **A 90-day feeding study in dogs is required**

9. **A 90-day inhalation toxicity study in rats is required**

D. MINORITY REPORTS

Sixteen "letters" from Brian Dementi, malathion toxicologist, either to Clark Swentzel, Chairman and /or to Jess Rowland, Executive Secretary of the Hazard Identification Assessment Review Committee are provided as Attachments 2 through 18. Attachment 1 contains the responses of the three external peer reviewers and Attachment 2 is the HIARC's report of December 17, 1997.

E. A summary of the doses, toxicology endpoints selected and Margins of Exposure (MOE) dietary and non-dietary exposure assessments are tabulated below..

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY	UF/ MOE
Acute Dietary	NOEL =50.0	Maternal toxicity	Range-Finding & Main Developmental toxicity studies - rabbits	UF =100
Chronic Dietary	NOEL=4.0	Inhibition of plasma cholinesterase activity	Combined/Chronic Toxicity Carcinogenicity - Rat	UF = 100
Short-Term (Dermal)	NOEL =50.0	Inhibition of plasma, RBC and brain cholinesterase activity	21-Day Dermal - Rabbit	MOE =100
Intermediate-Term (Dermal)	NOEL=50.0	Inhibition of plasma, RBC and brain cholinesterase activity	21-Day Dermal - Rabbit	MOE = 100
Long-Term (Dermal)	NOEL=4.0	Inhibition of plasma cholinesterase activity	Combined/ Chronic Toxicity - Rat	MOE = 100
Short-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBC cholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	MOE = 1000[a]
Intermediate-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBC cholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	MOE = 1000[a]
Long-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBC cholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	MOE = 1000[a]

a = A MOE of 1000 is required (includes the conventional 100 and an additional 10x for the use of a

LOEL and the severity of the nasal lesions observed in the two-week range finding study).

NOTE: The Aggregate Risk Index (ARI) should be used since different MOEs are required for dermal (MOE=100) and inhalation (MOE=1000) exposure risk assessments.

E. ATTACHMENTS

Attachment 1 Evaluations by the External Peer Review Members

Attachment 2 Report of the Hazard Identification Assessment Review Committee (12/17/97)

Attachment 3 Letter from Brian Dementi - November 10, 1997

Attachment 4 Letter from Brian Dementi - November 20, 1997

Attachment 5 Letter from Brian Dementi - November 25, 1997

Attachment 6 Letter from Brian Dementi - December 17, 1997

Attachment 7 Letter from Brian Dementi - January 15, 1998

Attachment 8 Letter from Brian Dementi - February 10, 1998

Attachment 9 Letter from Brian Dementi - March 10, 1998

Attachment 10 Letter from Brian Dementi - March 16, 1998

Attachment 11 Letter from Brian Dementi - March 20, 1998

Attachment 12 Letter from Brian Dementi - July 27, 1998

Attachment 13 Letter from Brian Dementi - July 29, 1998

Attachment 14 Letter from Brian Dementi - August 3, 1998

Attachment 15 Letter from Brian Dementi - August 10, 1998

Attachment 16 Letter from Brian Dementi - August 17, 1998

Attachment 17 Letter from Brian Dementi - September 24, 1998

Attachment 18 Letter from Brian Dementi - November 5, 1998